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I - C12Q1/68 ; C12N15/09

TI - GENOPOLYMORPHISM OF CONSTITUENT MASP-1 OF HUMAN COMPLEMENT-ACTIVATED LECTIN RaRF

AB - PROBLEM TO BE SOLVED: To provide a method for detecting a genopolymorphism involved in the onset of infectible diseases, particularly those in the babyhood.

- SOLUTION: This method for detecting a genopolymorphism involved in the onset of infectible diseases comprises amplifying a given region including the 148th base of the 6th intron of the constituent MASP-1 gene of human complement-activated lectin RaRF and detecting a mutation at the 148th base of the 6th intron. This method is useful for predicting the onset of infectible diseases, particularly those in the babyhood.

1/1 - (C) WPI / DERWENT
AN - 2002-552747 [59]
AP - JP20000356725 20001122
PR - JP20000356725 20001122
TI - Genetic polymorphism of MASP-1 which is a constituent of human complement-activating lectin RaRF, detection of genetic polymorphism related to outbreak of compromised diseases
IW - GENETIC POLYMORPH CONSTITUENT HUMAN COMPLEMENTARY ACTIVATE LECTIN DETECT GENETIC POLYMORPH RELATED OUTBREAK COMPROMISE DISEASE
PA - (KAWA-I) KAWAKAMI M
- (SUMU-N) SUMUKIN BIOSCIENCE KK
PN - JP2002153299A 20020528 DW200259 C12Q1/68 008pp
IC - C12N15/09 ; C12Q1/68
AB - JP2002153299 NOVELTY - Detecting genetic polymorphism related to outbreak of diseases comprising collecting a sample containing genomic DNA, amplifying a specific region containing the 148th base of the sixth intron of the gene of serine protease MASP-1 which is a constituent of human complement-activating lectin RaRF in a genomic DNA, and detecting a mutation in the base in the resultant amplified product, is new.
- DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an oligonucleotide primer set so that it can amplify a specific region containing the 148th base site in the sixth intron of the gene of serine protease MASP-1 which is a constituent of human complement-activating lectin RaRF based on the base sequence of the exon of MASP-1 gene and the base sequence of the sixth intron.
- USE - The method is used for detecting genetic polymorphism related to outbreak of compromised diseases.
- (Dwg.0/1)

* NOTICES *

CLAIMS

[Claim(s)]
[Claim 1] How to detect the gene polymorphism including the following process relevant to the onset of an opportunistic infection.
(1) Genomic DNA The process and (2) which extract the included sample Genomic DNA 148 of the 6th intron of the gene of serine protease MASP-1 which it sets and is the constituent of the Homo sapiens complement activity-ized lectin RaRF The process which carries out magnification processing of the fixed field containing the base of eye watch, and (3) It sets to the acquired magnification product and is 148 of the 6th intron of this MASP-1 gene. Process which detects the variation in the base of eye watch.
[Claim 2] The approach according to claim 1 an opportunistic infection is an opportunistic infection in infancy.
[Claim 3] The method according to claim 1 or 2 of performing magnification by the polymerase chain reaction method using the oligonucleotide primer set up based on the base sequence of the 6th exon of MASP-1 gene, and the base sequence of the 6th intron.
[Claim 4] The approach according to claim 3 using the reverse primer which has the forward primer which has the base sequence of the array number 3, and the base sequence of an array 4 as an oligonucleotide primer.
[Claim 5] About detection of variation, it is a single stranded DNA. The approach according to claim 1 to 4 of performing with a higher-order-structure polymorphism analysis method.
[Claim 6] 148 in the 6th intron of the gene of serine protease MASP-1 which is the component of the Homo sapiens complement activity-ized lectin RaRF based on the base sequence of the 6th exon of MASP-1 gene, and the base sequence of the 6th intron Oligonucleotide primer set up so that a fixed field including the base part of eye watch could be amplified.
[Claim 7] The oligonucleotide primer containing the reverse primer which has the base sequence of the forward primer which has the base sequence of the array number 3, and the array number 4 according to claim 6.
[Claim 8] The oligonucleotide primer according to claim 6 or 7 for detecting the gene polymorphism relevant to the onset of an opportunistic infection.
[Claim 9] The oligonucleotide primer according to claim 8 whose opportunistic infection is an opportunistic infection in infancy.

[Translation done.]

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention is Homo sapiens complement activity-ized lectin Ra-reactive factor (RaRF) more specifically relevant to the onset of an opportunistic infection, especially the opportunistic infection of infancy about the detection approach of gene polymorphism useful to the clinical laboratory test-diagnosis for expecting the onset of an opportunistic infection, especially the opportunistic infection of infancy. It is related with the primer used for the approach and detection which detect the gene polymorphism of complement activation component MASP-1.
[0002]
[Description of the Prior Art] The phylaxis by the lectin which is one of the phylaxis responses by devices other than an antibody production has attracted attention about the phylaxis to a pathogenic microorganism. Although the antibody is specific to each foreign matter, the role important for the biophylaxis of the infection first stage in front of an antibody production is played by always being produced, combining the complement activity-ized lectin RaRF which is the need and which increases by the way for a short time with the polysaccharide which exists in the front face of various microorganisms to the production taking fixed time amount, and activating complement. Moreover, becoming easy to be infected if variation takes place to the gene of the component protein of RaRF is found out.
[0003] It combines with the polysaccharide which exists in the front face of wide range microorganisms, such as a virus, bacteria, a fungus, and protozoa, and the complement activity-ized lectin RaRF which exists in the body fluid of vertebrates, such as Homo sapiens, guides an "MB lectin path" different from the conventional complement cascade, and induces protective reactions, such as an edible fungus, inflammation, and sterilization.
[0004] RaRF consists of a polysaccharide joint component which recognizes polysaccharide and is combined, and a complement activation component for activating complement. a polysaccharide joint component is called mannan joint lectin (mannan-binding lectin, MBL) -- having -- complement activity -- as a degassed part -- mannan joint lectin related serine protease (mannan-binding lectin associated serine protease, MASP) In addition to MASP-1 called and MASP-2, the compaction mold protein (small MBL-associated protein, sMAP) of MASP-2 with current [unknown / a function] is found out.
[0005] RaRF is MBL, although it is acute stage protein and increases for a short time at the time of inflammation. It increases also at the time of infection of bacteria or a virus, and reaching by the normal average [dozens times] is reported (Aittoniemi, J.et al., APMIS, 105:617-622, 1997). Also about MASP-1, it is MASP-1 mRNA at the time of inflammation. It is reported that the amount of manifestations increases several times. (Knittel, T.etal., Lab.Inv., 77:221-230, 1997) .
[0006] MBL which is the configuration protein of this RaRF Variation of a gene carries out the a large number report of causing various diseases including an opportunistic infection in recent years. Although it has so far considered as a weak constitution about the repetitive infection of slight illness that to infants seen, the abnormalities of complement activity-ized lectin attract attention as one of the leading factors leading to such an opportunistic infection. [many] For example, at the child who repeats a chronic digestive system disease and chronic otitis media, it is MBL in a blood serum. It was reported that there are many persons with low concentration [Super, M.et al., Lancet (ii), 1236-

1339, and 1989]. Behind, it is MBL. It is MBL if a gene varies. The phenomenon in which the serum concentration of protein becomes low is reported (Lipscombe, R.J.et al., Immunol.Lett., 32:253-257, 1992; Sumiya, M.et al., Lancet, 337:1569-1570, 1991; Super, M.et al., Nature Genet., 2:50-55, 1992). This is MBL. By the codon variation of a gene, it is Gly54 of a collagen region. Asp It is because it becomes and a structural change of the protein field starts. Variation Gly57 ->Glu of another site And Arg52 ->Cys But it is a blood serum MBL. It is reported that concentration falls.
[0007] Thus, MBL If a gene varies, it is the inside MBL of blood. Much clinical observation has shown coming to cause an infectious disease not only at reduction but at infancy repeatedly. In infancy, this has suggested that lectin has played the important role until it completes the immunity corresponding to the pathogenic microbe of varieties. Moreover, MBL When the both sides of homologue have gene variation, the example which an adult also causes a critical infectious disease is reported (Summerfield, J.A.et al., Lancet, 345:886-889, 1995). Furthermore, MBL A person with variation is HIV. If infected, the symptoms of AIDS are shown at an early stage, and it is reported that a life time is also short (Gerred, P.et al., Lancet, 349:236-240, 1997). Moreover, abnormalities It becomes easy for the person with MBL to start diseases other than an infectious disease. As the example, it is Gly54 ->Asp at serious illness atherosclerosis. Arg52 ->Cys Variation is Gly54 ->Asp at (Madsen, H.O.et al., Lancet, 352:959-960, 1998), and systemic lupus erythematosus. Variation is intentionally reported [many] (Lau Y.L.et al., Arthritis Rheum., 39:706-708, 1996).
[0008] Complement activation component MASP-1 which is another RaRF component is a serine protease component. MBL of RaRF If it combines with the polysaccharide of a microorganism, propagation activation of the information is carried out at MSAP, and the amino terminal side of the serine protease component of a MASP polypeptide is cut, it becomes an active type, and it is thought that this activates complement. Therefore, the variation of this gene reduces the drag force to infection, and is considered that diseases including an opportunistic infection arise. Therefore, detection of the gene variation of MASP-1 is very important when predicting diseases, such as an opportunistic infection.
[0009] MASP-1 gene is a long gene of 50,000 or more base pairs which exists in the 3rd chromosome macrobrachia of Homo sapiens, and consists of 16 or an exon beyond it (Takada, F.et al., Genomics, 25:757-759, 1995; Takayama, Y.et al., Mol.Immunol., 36:505-514, 1999). Although sequencing of the base sequence of this gene was carried out about the exon part, only the array of some fields was determined about the intron. Moreover, the exon of this gene and the polymorphism of the intron are not reported until now.
[0010] Usually, the variation of regulatory genes, such as a promotor who participates in the exon of a certain gene or a proteinic manifestation, is the variation of the intron of the near. (allotype) A chain is carried out closely in many cases. Since the variation of those genes is deeply concerned with production of protein, or the metergasia, diagnosing production of the protein or the disease caused by the metergasia, and its severity is performed by detecting this allotype.
[0011]
[Problem(s) to be Solved by the Invention] This invention aims at offering the approach of detecting the gene polymorphism of MASP-1 which is the constituent of RaRF which participates in the onset of an opportunistic infection, especially the opportunistic infection of infancy.
[0012]
[Means for Solving the Problem] this invention person etc. completed a header and this invention for the gene polymorphism of MASP-1 which is the complement activation component of RaRF which participates in the onset of an opportunistic infection, especially the opportunistic infection of infancy existing in the 6th intron part of this gene.
[0013] That is, this invention makes a summary the approach of detecting the gene polymorphism including the following process relevant to the onset of an opportunistic infection, especially the opportunistic infection of infancy. (1) Genomic DNA The process and (2) which extract the included sample Genomic DNA 148 of the 6th intron of the gene of serine protease MASP-1 which it sets and is the constituent of the Homo sapiens complement activity-ized lectin RaRF The process which carries out magnification processing of the fixed field containing the base of eye watch, and (3) It sets to the acquired magnification product and is 148 of the 6th intron of this MASP-1 gene. Process which detects the variation in the base of eye watch.

[0014] In the above-mentioned approach, it is desirable to perform magnification by the polymerase chain reaction method using the oligonucleotide primer set up based on the base sequence of the 6th exon of MASP-1 gene and the base sequence of the 6th intron, and it is desirable to use especially the reverse primer which has the forward primer which has the base sequence of the array number 3 as an oligonucleotide primer, and the base sequence of an array 4. Moreover, detection of variation is a single stranded DNA. It can carry out with a higher-order-structure polymorphism analysis method.
[0015] Furthermore, this invention is 148 in the 6th intron of the gene of serine protease MASP-1 which is the component of the Homo sapiens complement activation lectin RaRF based on the base sequence of the 6th exon of MASP-1 gene, and the base sequence of the 6th intron. It is related also with the oligonucleotide primer set up so that a fixed field including the base part of eye watch could be amplified. The primer pair containing the reverse primer which has the base sequence of the forward primer which has the base sequence of the array number 3, and the array number 4 as a suitable example of this oligonucleotide primer is mentioned. The oligonucleotide primer of this invention is useful although the gene polymorphism relevant to the onset of an opportunistic infection, especially the opportunistic infection of infancy is detected.
[0016] This invention detects the allotype of the gene of MASP-1 which is the important component of RaRF, and is MBL by detection of the allotype. It makes it possible to predict the opportunistic infection of an infectible body, especially infancy clinically like a case, and selection of a suitable cure is attained by it. Moreover, a diagnosis can be predicted also about diseases other than an infectious disease.
[0017]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. this invention person etc. analyzes the upper base sequence of the 6th intron of the gene of MASP-1 by sequencing of a dideoxy chain termination method, and is the 148th. It found out that the point mutation from a cytosine to a thymine existed in eye watch. The base sequence of a cytosine mold is shown in the array number 1, and the base sequence of a thymine mold is shown in the array number 2. He is Japanese 200 as the genotype determined by this point mutation is shown in the below-mentioned example. If people are judged, since the frequency of occurrence of the homozygote of the genotype which is a thymine is 6.5 %, allele will be judged to be polymorphism. It is thought that the approach of this invention enables it to predict the onset of an opportunistic infection.
[0018] Thus, the polymorphism in the 6th intron exists in the gene of MASP-1 which is the component of RaRF which participates in the onset of an opportunistic infection, especially the opportunistic infection of infancy, and in this invention, as detection of this polymorphism is explained to a detail, it is performed to below.
[0019] The detection approach of the gene polymorphism in this invention, (1) genomic DNA The process and (2) which extract the included sample Genomic DNA It sets. Serine protease which is the constituent of the Homo sapiens complement activity-ized lectin RaRF MASP-1 148 of the 6th intron of a gene The process which carries out magnification processing of the fixed field containing the base of eye watch, and (3) In the acquired magnification product ** MASP-1 148 of the 6th intron of a gene The process which detects the variation in the base of eye watch is included.
[0020] It sets to this invention approach and is genomic DNA. It considers as the measuring object and is this DNA. It can adopt without being limited if it is the sample of the Homo sapiens origin as an ingredient for preparing. For example, a conventional method is followed from samples, such as tissue cells, such as a cell of body fluid, such as blood, bone marrow liquid, sperm, and abdominal cavity liquid, tunica mucosa oris, and liver, and a hair root cell adhering to hair, and it is genomic DNA. What is necessary is to extract and just to refine.
[0021] Prepared genomic DNA In order to use and to detect the variation of the 6th intron of MASP-1 gene, it is ** of this intron first. DNA including the 148th variation part DNA which amplified the field A fragment is produced. DNA Magnification is for example, the polymerase chain reaction method. (PCR law) It can follow and carry out and the primer suitably set up so that the fixed field which includes the variation part of the 6th intron of the above in that case might be amplified specifically is used. A primer is the base sequence of the 6th exon of MASP-1 gene. (array number 5) And magnification DNA of dozens which include the variation part in the 6th intron of MASP-1

gene based on the base sequence of the 6th intron to about hundreds base pair What is necessary is just to set up so that it may be obtained. Obtained magnification DNA Single stranded DNA When analyzing with a higher-order-structure polymorphism analysis method, it is 300-500. Magnification DNA of base pair extent Obtaining is desirable. Moreover, DNA As for the die length of the primer used for magnification, it is desirable to consider as 18 - 25 base extent.
[0022] It is such. The following desirable primers are mentioned as a primer used by the PCR method.
forward primer: -- 5 -- '-GGGCCTTTCTGTGGAGAGAA-3' -- reverse (array number 3) primer: -- 5'-AATCTCTCTATGCGATACTGAA-3' PCR method (array number 4) etc. -- DNA the need after amplifying -- responding -- PCR A column etc. removes the superfluous primer and superfluous nucleotide in a product.
[0023] Next, DNA which carried out magnification processing as mentioned above A fragment is used and it is ** of the 6th intron of MASP-1. Although the 148th variation is detected, the approach of arbitration can be used without being restricted in any way as the detection approach of variation. For example, DNA which can be used by this invention As a variation detecting method in a fragment Single stranded DNA Higher-order-structure polymorphism (single-stranded conformation polymorphism, SSCP) Analysis method, Restriction enzyme fragment length polymorphism (restriction fragmentlength polymorphism, RFLP) Analysis method, Heteroduplex analysis (heteroduplex analysis, HET) Law, Modifier concentration gradient gel electrophoresis (denaturing gradient gel electrophoresis, DGGE) Law, Direct sequencing (direct sequence, DS) Law, carbodiimide qualification (carbodiimide modification, CDI) Law etc. is mentioned. [biotechnology manual series 1, the basic technique of gene engineering, Yamamoto elegant editing, Yodosha (1993) referring-to-] .
[0024] (In addition, the various actuation which may be adopted in the variation detecting method of this invention, for example, DNA, Chemosynthesis and DNA Each of isolation, purification, magnification, selections, etc. should just follow a conventional method.) For example, DNA Being able to perform isolation purification with agarose gel electrophoresis etc., the decision of an array is a dideoxy chain termination method (Sanger, F.et al., Proc.Natl.Acad.Sci., U.S.A., 74:5463-5467, 1977). The Maxam-gilbert method (Maxam, A.M.et al., Method inEnzymology, 65:499-560, 1980) etc. can be used. DNA The decision of a base sequence may be made using a commercial sequence kit etc. DNA PCR for amplifying a specific region Law is also a conventional method. (for example, Saiki, R.K.et al., Science, 230:1350-1354, 1985) It can carry out by following.
[0025] DNA Because of detection of a fragment, it is DNA. The indicator of the fragment can also be carried out with the following matter. DNA As an approach of carrying out the indicator of the fragment, it is the approach and PCR which carry out the indicator of the primer beforehand. Base component which carried out the indicator when carrying out (for example, radioactive indicator of Lynn) The approach of using, or PCR After carrying out, the approach of carrying out an indicator is.
[0026] the matter which can be used for an indicator -- especially -- a limit -- there is nothing -- a radioactive substance, a fluorescent material, the chemiluminescence matter, and biotin (it detects by enzyme-labeling avidin) etc. -- it is -- for example, DNA What is necessary is just to carry out the indicator of the five prime end side. It is PCR especially preferably. It is Cy5 beforehand to the five prime end of the oligonucleotide primer of **. What carried out fluorescent labeling (Amersham Pharmacia Biotech K.K.) It is used. What applied this fluorescent-labeling approach to the SSCP method is called fluorescence SSCP method.
[0027] In order to detect variation in this invention, it is a single stranded DNA suitably. Higher-order-structure polymorphism analysis method (the SSCP method) Or restriction enzyme fragment length polymorphism analysis method (RFLP law) It is employable. Especially, it is PCR. The approach which combined the magnification technique and the describing [above] fluorescence SSCP method by law is desirable. This approach is PCR. DNA using law Since it is combination with the magnification technique, it is little DNA. Easy moreover, simple and high detection of sensibility and precision are possible using a sample. Moreover, the suitable conditions which perform the SSCP method are about 6% acrylamide especially, although it is 15-25 degrees C in 6 - 8% acrylamide gel, and migration temperature. (acrylamide: bis-acrylamide = 99:1) If it carries out

at the migration temperature of about 20 degrees C using gel, it is advantageous in respect of exact detection of polymorphism.
[0028] the following examples -- setting -- fluorescence PCR-SSCP -- using -- genomic DNA ******** -- although the example which detects the variation in the 6th intron of MASP-1 gene is explained, without it restricts to this detection approach -- RFLP -- the detection approach of arbitration including law can be used.
[0029]
[Example] Although the following examples explain this invention in more detail, this invention is not limited to this example.
[0030] The reagent used in this example is as being shown below.
<40% acrylamide preservation liquid> acrylamide 79.2g and NN'-methylenebis acrylamide 0.8 g are dissolved with purified water, it is referred to as 200 ml, Amberlite 10g is added, and it shakes slowly in the state of protection from light overnight. 4 degrees C is saved in the state of protection from light.
<20xTME (6.2)> tris 36.3g, MES 115.2 g, and EDTA 3.7g are dissolved with purified water, and it is referred to as 500 ml.
It is purified water 60 ml to <6% acrylamide gel> 40% acrylamide preservation liquid 11.25 ml and 20xTME(6.2) 3.75 ml. It is the 10% ammonium persulfate 400 after filter filtration degassing moreover. mul and TEMED 40microl It adds.
[0031] (1) which explains below the experiment actuation used in this example Homo sapiens peripheral blood genomic DNA It is genomic DNA from preparation Homo sapiens peripheral blood. SepaGene (Sanko Junyaku, Inc.) It uses and prepares according to the approach of the following according to a kit usage.
[0032] Homo sapiens peripheral blood 100 which collected blood using anticoagulant EDTA-2Na mul It puts into the micro tube of 1.5 ml **, and is the reagent I of a SepaGene kit (specimen diluent). 100microl In addition, it mixes by the mixer and puts at a room temperature for 10 minutes. Subsequently, reagent II (pretreatment liquid) of this kit 100microl It is the reagent III (extractant) of after mixing and this kit lightly with a mixer moreover. 700 mul and reagent IV400(extractant) microl of this kit In addition, it stirs violently 10 times. The at-long-intervals alignment of this is carried out at 12,000 rotations and 4 degrees C for 15 minutes, and it is supernatant liquid 500. mul It puts into the micro tube of new 1.5 ml **. Reagent V of coprecipitater 2microl and this kit (precipitate adjuvant) 50microl, isopropanol 600microl In addition, it puts for 15 minutes in -70 degrees C after fall mixing, and supernatant liquid is removed after a 20-minute alignment at long intervals at 15,000 rotations and 4 degrees C. 70% ethanol 500microl In addition, an at-long-intervals alignment is lightly carried out at 15,000 rotations and 4 degrees C after fall mixing for 5 minutes, and supernatant liquid is removed. 100 % ethanol 500microl In addition, an at-long-intervals alignment is lightly carried out at 15,000 rotations and 4 degrees C after fall mixing for 5 minutes, and supernatant liquid is removed. It is 50microl after an air dried and about sterile distilled water in precipitate. It adds.
[0033] (2) DNA The magnification actuation above (1) Prepared genomic DNA Solution (50-100 ng/mu l) 1microl Sterile-distilled-water 37.5microl and 10xKOD Dash PCR solution 5microl, 2mMdNTPs(es) (four sorts of deoxyribonucleoside triphosphoric acid) 5microl, 20microM Forward primer 0.5 mul and 20microM Reverse primer 0.5 mul, 2.5 U/mu l KOD Dash 0.5microl adding -- the whole quantity -- 50microl ** -- carry out, carry out two-drop multistory [of the mineral oil] further, and let 74-degree-C 1 minute be 1 cycle for 60-degree-C 10 seconds for 94-degree-C 30 seconds 35 cycle is reacted. PCR The ** primer has the following base sequence. These primers are a five prime end Cy5 An indicator is carried out.
<oligonucleotide primer> forward primer: -- 5 -- '-GGGCCTTTCTGTGGAGAGAA-3' -- reverse (array number 3) primer: -- 5'-AATCTCTCTATGCGATACTGAA-3' PCR (array number 4) After electrophoresis and the ethidium bromide dye the check of a product by agarose gel 3%, and it is performed.
[0034] (3) Superfluous primer removal actuation PCR In order to remove the superfluous primer in a product, it is MicroSpin S-300 HR Column (Amersham Pharmacia Biotech K.K.). It uses and the following actuation is performed according to column directions for use.

[0035] First, PCR which has carried out multistory [of the mineral oil] It is chloroform 50microl to a product. In addition, by the mixer, after mixing, centrifugal is carried out and a mineral oil is removed. The resin with which the column is filled up is put into the micro tube of 2.0 ml ** after mixing by the mixer, and it is 3,000. Except for the excessive buffer solution which carried out the at-long-intervals alignment by rotation for 1 minute and which had been added in the column. PCR put this column into the micro tube of new 1.5 ml **, and excluding the mineral oil A product is added and it is 3,000. An at-long-intervals alignment is carried out by rotation for 2 minutes.
[0036] (4) SSCP actuation fluorescence SSCP analysis is A.L.F.express DNA sequencer (Amersham FAARUMASHIA biotechnology theque company) which attached the external cooling circulation tank. 6% acrylamide which uses it and contains the 1xTME (6.2) buffer solution (acrylamide: bis-acrylamide = 99:1) Gel (height 270 mmx width-of-face 308 mm x thickness 0.5 mm) It uses. PCR which removed the superfluous primer and the nucleotide 3microl of a product Loading liquid 3microl for SSCP It is 2microl after thermal denaturation and of these for 5 minutes at 95 degrees C moreover. It applies to gel and migrates. The presentation of loading liquid is the formamide solution containing Blue Dextran 0.05% which carried out deionization processing. The migration buffer solution used 1xTME (6.2). In the analysis of measurement data, it is the software Fragment Manager V1.2 (Amersham Pharmacia Biotech K.K.) for analysis. It uses.
[0037] (5) as the SSCP electrophoresis condition electrophoresis temperature of 20 degrees C, electrical-potential-difference 1500V, 100mA of currents, power 30W, and the migration buffer solution -- 1xTME (6.2) -- using it -- 500 a part -- between -- electrophoresis -- it carries out.
[0038] (6) Genotype judging PCR DNA obtained by carrying out magnification processing It is the software Fragment Manager V1.2 (Amersham Pharmacia Biotech K.K.) for analysis about the peak which carried out thermal denaturation of the fragment and was separated according to the difference of the mobility of single-stranded higher order structure. It uses and analyzes and genotype is judged. the judgment of genotype -- drawing 1 .A the case where a pattern is detected -- 148 in the 6th intron the base of eye watch -- the homozygote of a cytosine, and drawing 1 .B the case where a pattern is detected -- the heterozygote of a cytosine and a thymine, and drawing 1 .C a pattern -- detection **** -- a case -- a thymine -- it considers as a homozygote. The check of a base sequence is Thermo Sequenase Cy5 Dye Terminator Kit (Amersham Pharmacia Biotech K.K.). The used base sequence analysis performs. Under the present circumstances, 5'-TTCCATAGTGACAACTCGGG-3' is used for an oligonucleotide primer.
[0039] [Example 1] This example was performed according to each actuation of the above-mentioned experiment actuation. Adult 200 of the Japanese who chose at random About a name, it is the above-mentioned experiment actuation (1). - (6) It follows and is genomic DNA. Variation by preparation, magnification processing, removal of a superfluous primer, and SSCP was detected, and genotype was judged. The frequency of occurrence of each genotype is shown in Table 1. In addition, the 148th of the 6th intron of MASP-1 gene Allele wrote [the genotype of the homozygote whose watch base is a cytosine] TT [the genotype of TC and the homozygote which are both thymines] for the genotype of CC and the heterozygote which are a cytosine and a thymine, respectively.
[0040] [Table 1] 200 MASP-1 genotype frequency-of-occurrence analyses in healthy people CC TC TT number (frequency) 116 (58.0%) 71 (35.5%) 13 (6.5%) [0041] 58.0%, the genotype TT of the homozygote which is a thymine 35.5% is 6.5 %, therefore both the genotypes TC of the heterozygote which are a cytosine and a thymine, respectively are [the genotype CC of the homozygote whose allele is a cytosine for a result] the 148th of the 6th intron of MASP-1 gene. The variation from the cytosine in a watch base to a thymine is judged to be polymorphism.
[0042]
[Effect of the Invention] According to this invention, the method of analyzing the gene polymorphism relevant to an opportunistic infection, especially the opportunistic infection of infancy, i.e., the gene polymorphism of constituent MASP-1 of the Homo sapiens complement activity-ized lectin RaRF, is offered. By detection of this polymorphism, the anticipation diagnosis of an opportunistic infection, especially the opportunistic infection of infancy may be able to be carried out clinically, and selection of the cure for those diseases is attained by this diagnosis.

[Translation done.]

* NOTICES *

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TECHNICAL FIELD

[Field of the Invention] This invention is Homo sapiens complement activity-ized lectin Ra-reactive factor (RaRF) more specifically relevant to the onset of an opportunistic infection, especially the opportunistic infection of infancy about the detection approach of gene polymorphism useful to the clinical laboratory test-diagnosis for expecting the onset of an opportunistic infection, especially the opportunistic infection of infancy. It is related with the primer used for the approach and detection which detect the gene polymorphism of complement activation component MASP-1.
[0002]

[Translation done.]

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PRIOR ART

[Description of the Prior Art] The phylaxis by the lectin which is one of the phylaxis responses by devices other than an antibody production has attracted attention about the phylaxis to a pathogenic microorganism. Although the antibody is specific to each foreign matter, the role important for the biophylaxis of the infection first stage in front of an antibody production is played by always being produced, combining the complement activity-ized lectin RaRF which is the need and which increases by the way for a short time with the polysaccharide which exists in the front face of various microorganisms to the production taking fixed time amount, and activating complement. Moreover, becoming easy to be infected if variation takes place to the gene of the component protein of RaRF is found out.
[0003] It combines with the polysaccharide which exists in the front face of wide range microorganisms, such as a virus, bacteria, a fungus, and protozoa, and the complement activity-ized lectin RaRF which exists in the body fluid of vertebrates, such as Homo sapiens, guides an "MB lectin path" different from the conventional complement cascade, and induces protective reactions, such as an edible fungus, inflammation, and sterilization.
[0004] RaRF consists of a polysaccharide joint component which recognizes polysaccharide and is combined, and a complement activation component for activating complement. a polysaccharide joint component is called mannan joint lectin (mannan-binding lectin, MBL) -- having -- complement activity -- as a degassed part -- mannan joint lectin related serine protease (mannan-binding lectin associated serine protease, MASP) In addition to MASP-1 called and MASP-2, the compaction mold protein (small MBL-associated protein, sMAP) of MASP-2 with current [unknown / a function] is found out.
[0005] RaRF is MBL, although it is acute stage protein and increases for a short time at the time of inflammation. It increases also at the time of infection of bacteria or a virus, and reaching by the normal average [dozens times] is reported (Aittoniemi, J.et al., APMIS, 105:617-622, 1997). Also about MASP-1, it is MASP-1 mRNA at the time of inflammation. It is reported that the amount of manifestations increases several times. (Knittel, T.etal., Lab.Inv., 77:221-230, 1997).
[0006] MBL which is the configuration protein of this RaRF Variation of a gene carries out the a large number report of causing various diseases including an opportunistic infection in recent years. Although it has so far considered as a weak constitution about the repetitive infection of slight illness that to infants seen, the abnormalities of complement activity-ized lectin attract attention as one of the leading factors leading to such an opportunistic infection. [many] For example, at the child who repeats a chronic digestive system disease and chronic otitis media, it is MBL in a blood serum. It was reported that there are many persons with low concentration [Super, M.et al., Lancet (ii), 1236-1339, and 1989]. Behind, it is MBL. It is MBL if a gene varies. The phenomenon in which the serum concentration of protein becomes low is reported (Lipscombe, R.J.et al., Immunol.Lett., 32:253-257, 1992; Sumiya, M.et al., Lancet, 337:1569-1570, 1991; Super, M.et al., Nature Genet., 2:50-55, 1992). This is MBL. By the codon variation of a gene, it is Gly54 of a collagen region. Asp It is because it becomes and a structural change of the protein field starts. Variation Gly57 ->Glu of another site And Arg52 ->Cys But it is a blood serum MBL. It is reported that concentration falls.
[0007] Thus, MBL If a gene varies, it is the inside MBL of blood. Much clinical observation has shown coming to cause an infectious disease not only at reduction but at infancy repeatedly. In infancy, this has suggested that lectin has played the important role until it completes the immunity

corresponding to the pathogenic microbe of varieties. Moreover, MBL When the both sides of homologue have gene variation, the example which an adult also causes a critical infectious disease is reported (Summerfield, J.A.et al., Lancet, 345:886-889, 1995). Furthermore, MBL A person with variation is HIV. If infected, the symptoms of AIDS are shown at an early stage, and it is reported that a life time is also short (Gerred, P.et al., Lancet, 349:236-240, 1997). Moreover, abnormalities It becomes easy for the person with MBL to start diseases other than an infectious disease. As the example, it is Gly54 ->Asp at serious illness atherosclerosis. Arg52 ->Cys Variation is Gly54 ->Asp at (Madsen, H.O.et al., Lancet, 352:959-960, 1998), and systemic lupus erythematosus. Variation is intentionally reported [many] (Lau Y.L.et al., Arthritis Rheum., 39:706-708, 1996).

[0008] Complement activation component MASP-1 which is another RaRF component is a serine protease component. MBL of RaRF If it combines with the polysaccharide of a microorganism, propagation activation of the information is carried out at MSAP, and the amino terminal side of the serine protease component of a MASP polypeptide is cut, it becomes an active type, and it is thought that this activates complement. Therefore, the variation of this gene reduces the drag force to infection, and is considered that diseases including an opportunistic infection arise. Therefore, detection of the gene variation of MASP-1 is very important when predicting diseases, such as an opportunistic infection.

[0009] MASP-1 gene is a long gene of 50,000 or more base pairs which exists in the 3rd chromosome macrobrachia of Homo sapiens, and consists of 16 or an exon beyond it (Takada, F.et al., Genomics, 25:757-759, 1995; Takayama, Y.et al., Mol.Immunol., 36:505-514, 1999). Although sequencing of the base sequence of this gene was carried out about the exon part, only the array of some fields was determined about the intron. Moreover, the exon of this gene and the polymorphism of the intron are not reported until now.

[0010] Usually, the variation of regulatory genes, such as a promotor who participates in the exon of a certain gene or a proteinic manifestation, is the variation of the intron of the near. (allotype) A chain is carried out closely in many cases. Since the variation of those genes is deeply concerned with production of protein, or the metergasia, diagnosing production of the protein or the disease caused by the metergasia, and its severity is performed by detecting this allotype.

[Translation done.]

* NOTICES *

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EFFECT OF THE INVENTION

[Effect of the Invention] According to this invention, the method of analyzing the gene polymorphism relevant to an opportunistic infection, especially the opportunistic infection of infancy, i.e., the gene polymorphism of constituent MASP-1 of the Homo sapiens complement activity-ized lectin RaRF, is offered. By detection of this polymorphism, the anticipation diagnosis of an opportunistic infection, especially the opportunistic infection of infancy may be able to be carried out clinically, and selection of the cure for those diseases is attained by this diagnosis.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] This invention aims at offering the approach of detecting the gene polymorphism of MASP-1 which is the constituent of RaRF which participates in the onset of an opportunistic infection, especially the opportunistic infection of infancy.

[Translation done.]

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MEANS

[Means for Solving the Problem] this invention person etc. completed a header and this invention for the gene polymorphism of MASP-1 which is the complement activation component of RaRF which participates in the onset of an opportunistic infection, especially the opportunistic infection of infancy existing in the 6th intron part of this gene.
[0013] That is, this invention makes a summary the approach of detecting the gene polymorphism including the following process relevant to the onset of an opportunistic infection, especially the opportunistic infection of infancy. (1) Genomic DNA The process and (2) which extract the included sample Genomic DNA 148 of the 6th intron of the gene of serine protease MASP-1 which it sets and is the constituent of the Homo sapiens complement activity-ized lectin RaRF The process which carries out magnification processing of the fixed field containing the base of eye watch, and (3) It sets to the acquired magnification product and is 148 of the 6th intron of this MASP-1 gene. Process which detects the variation in the base of eye watch.
[0014] In the above-mentioned approach, it is desirable to perform magnification by the polymerase chain reaction method using the oligonucleotide primer set up based on the base sequence of the 6th exon of MASP-1 gene and the base sequence of the 6th intron, and it is desirable to use especially the reverse primer which has the forward primer which has the base sequence of the array number 3 as an oligonucleotide primer, and the base sequence of an array 4. Moreover, detection of variation is a single stranded DNA. It can carry out with a higher-order-structure polymorphism analysis method.
[0015] Furthermore, this invention is 148 in the 6th intron of the gene of serine protease MASP-1 which is the component of the Homo sapiens complement activation lectin RaRF based on the base sequence of the 6th exon of MASP-1 gene, and the base sequence of the 6th intron. It is related also with the oligonucleotide primer set up so that a fixed field including the base part of eye watch could be amplified. The primer pair containing the reverse primer which has the base sequence of the forward primer which has the base sequence of the array number 3, and the array number 4 as a suitable example of this oligonucleotide primer is mentioned. The oligonucleotide primer of this invention is useful although the gene polymorphism relevant to the onset of an opportunistic infection, especially the opportunistic infection of infancy is detected.
[0016] This invention detects the allotype of the gene of MASP-1 which is the important component of RaRF, and is MBL by detection of the allotype. It makes it possible to predict the opportunistic infection of an infectible body, especially infancy clinically like a case, and selection of a suitable cure is attained by it. Moreover, a diagnosis can be predicted also about diseases other than an infectious disease.
[0017]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. this invention person etc. analyzes the upper base sequence of the 6th intron of the gene of MASP-1 by sequencing of a dideoxy chain termination method, and is the 148th. It found out that the point mutation from a cytosine to a thymine existed in eye watch. The base sequence of a cytosine mold is shown in the array number 1, and the base sequence of a thymine mold is shown in the array number 2. He is Japanese 200 as the genotype determined by this point mutation is shown in the below-mentioned example. If people are judged, since the frequency of occurrence of the homozygote of the genotype which is a thymine is 6.5 %, allele will be judged to be polymorphism. It is thought that the approach

of this invention enables it to predict the onset of an opportunistic infection.
[0018] Thus, the polymorphism in the 6th intron exists in the gene of MASP-1 which is the component of RaRF which participates in the onset of an opportunistic infection, especially the opportunistic infection of infancy, and in this invention, as detection of this polymorphism is explained to a detail, it is performed to below.
[0019] The detection approach of the gene polymorphism in this invention, (1) genomic DNA The process and (2) which extract the included sample Genomic DNA It sets. Serine protease which is the constituent of the Homo sapiens complement activity-ized lectin RaRF MASP-1 148 of the 6th intron of a gene The process which carries out magnification processing of the fixed field containing the base of eye watch, and (3) In the acquired magnification product ** MASP-1 148 of the 6th intron of a gene The process which detects the variation in the base of eye watch is included.
[0020] It sets to this invention approach and is genomic DNA. It considers as the measuring object and is this DNA. It can adopt without being limited if it is the sample of the Homo sapiens origin as an ingredient for preparing. For example, a conventional method is followed from samples, such as tissue cells, such as a cell of body fluid, such as blood, bone marrow liquid, sperm, and abdominal cavity liquid, tunica mucosa oris, and liver, and a hair root cell adhering to hair, and it is genomic DNA. What is necessary is to extract and just to refine.
[0021] Prepared genomic DNA In order to use and to detect the variation of the 6th intron of MASP-1 gene, it is ** of this intron first. DNA including the 148th variation part DNA which amplified the field A fragment is produced. DNA Magnification is for example, the polymerase chain reaction method. (PCR law) It can follow and carry out and the primer suitably set up so that the fixed field which includes the variation part of the 6th intron of the above in that case might be amplified specifically is used. A primer is the base sequence of the 6th exon of MASP-1 gene. (array number 5) And magnification DNA of dozens which include the variation part in the 6th intron of MASP-1 gene based on the base sequence of the 6th intron to about hundreds base pair What is necessary is just to set up so that it may be obtained. Obtained magnification DNA Single stranded DNA When analyzing with a higher-order-structure polymorphism analysis method, it is 300-500. Magnification DNA of base pair extent Obtaining is desirable. Moreover, DNA As for the die length of the primer used for magnification, it is desirable to consider as 18 - 25 base extent.
[0022] It is such. The following desirable primers are mentioned as a primer used by the PCR method.
forward primer: -- 5 -- '-GGGCCTTTCTGTGGAGAGAA-3' -- reverse (array number 3) primer: -- 5'-AATCTCTCTATGCGATACTGAA-3' PCR method (array number 4) etc. -- DNA the need after amplifying -- responding -- PCR A column etc. removes the superfluous primer and superfluous nucleotide in a product.
[0023] Next, DNA which carried out magnification processing as mentioned above A fragment is used and it is ** of the 6th intron of MASP-1. Although the 148th variation is detected, the approach of arbitration can be used without being restricted in any way as the detection approach of variation. For example, DNA which can be used by this invention As a variation detecting method in a fragment Single stranded DNA Higher-order-structure polymorphism (single-stranded conformation polymorphism, SSCP) Analysis method, Restriction enzyme fragment length polymorphism (restriction fragmentlength polymorphism, RFLP) Analysis method, Heteroduplex analysis (heteroduplex analysis, HET) Law, Modifier concentration gradient gel electrophoresis (denaturing gradient gel electrophoresis, DGGE) Law, Direct sequencing (direct sequence, DS) Law, carbodiimide qualification (carbodiimide modification, CDI) Law etc. is mentioned. [biotechnology manual series 1, the basic technique of gene engineering, Yamamoto elegant editing, Yodosha (1993) referring-to-] :
[0024] (In addition, the various actuation which may be adopted in the variation detecting method of this invention, for example, DNA, Chemosynthesis and DNA Each of isolation, purification, magnification, selections, etc. should just follow a conventional method.) For example, DNA Being able to perform isolation purification with agarose gel electrophoresis etc., the decision of an array is a dideoxy chain termination method (Sanger, F.et al., Proc.Natl.Acad.Sci., U.S.A., 74:5463-5467, 1977). The Maxam-gilbert method (Maxam, A.M.et al., Method inEnzymology, 65:499-560, 1980) etc. can be used. DNA The decision of a base sequence may be made using a commercial sequence

kit etc. DNA PCR for amplifying a specific region Law is also a conventional method. (for example, Saiki, R.K.et al., Science, 230:1350-1354, 1985) It can carry out by following.
[0025] DNA Because of detection of a fragment, it is DNA. The indicator of the fragment can also be carried out with the following matter. DNA As an approach of carrying out the indicator of the fragment, it is the approach and PCR which carry out the indicator of the primer beforehand. Base component which carried out the indicator when carrying out (for example, radioactive indicator of Lynn) The approach of using, or PCR After carrying out, the approach of carrying out an indicator is.
[0026] the matter which can be used for an indicator -- especially -- a limit -- there is nothing -- a radioactive substance, a fluorescent material, the chemiluminescence matter, and biotin (it detects by enzyme-labeling avidin) etc. -- it is -- for example, DNA What is necessary is just to carry out the indicator of the five prime end side. It is PCR especially preferably. It is Cy5 beforehand to the five prime end of the oligonucleotide primer of **. What carried out fluorescent labeling (Amersham Pharmacia Biotech K.K.) It is used. What applied this fluorescent-labeling approach to the SSCP method is called fluorescence SSCP method.
[0027] In order to detect variation in this invention, it is a single stranded DNA suitably. Higher-order-structure polymorphism analysis method (the SSCP method) Or restriction enzyme fragment length polymorphism analysis method (RFLP law) It is employable. Especially, it is PCR. The approach which combined the magnification technique and the describing [above] fluorescence SSCP method by law is desirable. This approach is PCR. DNA using law Since it is combination with the magnification technique, it is little DNA. Easy moreover, simple and high detection of sensibility and precision are possible using a sample. Moreover, the suitable conditions which perform the SSCP method are about 6% acrylamide especially, although it is 15-25 degrees C in 6 - 8% acrylamide gel, and migration temperature. (acrylamide: bis-acrylamide = 99:1) If it carries out at the migration temperature of about 20 degrees C using gel, it is advantageous in respect of exact detection of polymorphism.
[0028] the following examples -- setting -- fluorescence PCR-SSCP -- using -- genomic DNA ******** -- although the example which detects the variation in the 6th intron of MASP-1 gene is explained, without it restricts to this detection approach -- RFLP -- the detection approach of arbitration including law can be used.

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EXAMPLE

[Example] Although the following examples explain this invention in more detail, this invention is not limited to this example.
[0030] The reagent used in this example is as being shown below.
<40% acrylamide preservation liquid> acrylamide 79.2g and NN'-methylenebis acrylamide 0.8 g are dissolved with purified water, it is referred to as 200 ml, Amberlite 10g is added, and it shakes slowly in the state of protection from light overnight. 4 degrees C is saved in the state of protection from light.
<20xTME (6.2)> tris 36.3g, MES 115.2 g, and EDTA 3.7g are dissolved with purified water, and it is referred to as 500 ml.
It is purified water 60 ml to <6% acrylamide gel> 40% acrylamide preservation liquid 11.25 ml and 20xTME(6.2) 3.75 ml. It is the 10% ammonium persulfate 400 after filter filtration degassing moreover. mul and TEMED 40microl It adds.
[0031] (1) which explains below the experiment actuation used in this example Homo sapiens peripheral blood genomic DNA It is genomic DNA from preparation Homo sapiens peripheral blood. SepaGene (Sanko Junyaku, Inc.) It uses and prepares according to the approach of the following according to a kit usage.
[0032] Homo sapiens peripheral blood 100 which collected blood using anticoagulant EDTA-2Na mul It puts into the micro tube of 1.5 ml **, and is the reagent I of a SepaGene kit (specimen diluent). 100microl In addition, it mixes by the mixer and puts at a room temperature for 10 minutes. Subsequently, reagent II (pretreatment liquid) of this kit 100microl It is the reagent III (extractant) of after mixing and this kit lightly with a mixer moreover. 700 mul and reagent IV400(extractant) microl of this kit In addition, it stirs violently 10 times. The at-long-intervals alignment of this is carried out at 12,000 rotations and 4 degrees C for 15 minutes, and it is supernatant liquid 500. mul It puts into the micro tube of new 1.5 ml **. Reagent V of coprecipitater 2microl and this kit (precipitate adjuvant) 50microl, isopropanol 600microl In addition, it puts for 15 minutes in -70 degrees C after fall mixing, and supernatant liquid is removed after a 20-minute alignment at long intervals at 15,000 rotations and 4 degrees C. 70% ethanol 500microl In addition, an at-long-intervals alignment is lightly carried out at 15,000 rotations and 4 degrees C after fall mixing for 5 minutes, and supernatant liquid is removed. 100 % ethanol 500microl In addition, an at-long-intervals alignment is lightly carried out at 15,000 rotations and 4 degrees C after fall mixing for 5 minutes, and supernatant liquid is removed. It is 50microl after an air dried and about sterile distilled water in precipitate. It adds.
[0033] (2) DNA The magnification actuation above (1) Prepared genomic DNA Solution (50-100 ng/mu l) 1microl Sterile-distilled-water 37.5microl and 10xKOD Dash PCR solution 5microl, 2mMdNTPs(es) (four sorts of deoxyribonucleoside triphosphoric acid) 5microl, 20microM Forward primer 0.5 mul and 20microM Reverse primer 0.5 mul, 2.5 U/mu l KOD Dash 0.5microl adding -- the whole quantity -- 50microl ** -- carry out, carry out two-drop multistory [of the mineral oil] further, and let 74-degree-C 1 minute be 1 cycle for 60-degree-C 10 seconds for 94-degree-C 30 seconds 35 cycle is reacted. PCR The ** primer has the following base sequence. These primers are a five prime end Cy5 An indicator is carried out.
<oligonucleotide primer> forward primer: -- 5 -- '-GGGCCTTTCTGTGGAGAGAA-3' -- reverse (array number 3) primer: -- 5'-AATCTCTCTATGCGATACTGAA-3' PCR (array number 4) After

electrophoresis and the ethidium bromide dye the check of a product by agarose gel 3%, and it is performed.
[0034] (3) Superfluous primer removal actuation PCR In order to remove the superfluous primer in a product, it is MicroSpin S-300 HR Column (Amersham Pharmacia Biotech K.K.). It uses and the following actuation is performed according to column directions for use.
[0035] First, PCR which has carried out multistory [of the mineral oil] It is chloroform 50microl to a product. In addition, by the mixer, after mixing, centrifugal is carried out and a mineral oil is removed. The resin with which the column is filled up is put into the micro tube of 2.0 ml ** after mixing by the mixer, and it is 3,000. Except for the excessive buffer solution which carried out the at-long-intervals alignment by rotation for 1 minute and which had been added in the column. PCR put this column into the micro tube of new 1.5 ml **, and excluding the mineral oil A product is added and it is 3,000. An at-long-intervals alignment is carried out by rotation for 2 minutes.
[0036] (4) SSCP actuation fluorescence SSCP analysis is A.L.F.express DNA sequencer (Amersham FAARUMASHIA biotechnology theque company) which attached the external cooling circulation tank. 6% acrylamide which uses it and contains the 1xTME (6.2) buffer solution (acrylamide: bis-acrylamide = 99:1) Gel (height 270 mmx width-of-face 308 mm x thickness 0.5 mm) It uses. PCR which removed the superfluous primer and the nucleotide 3microl of a product Loading liquid 3microl for SSCP It is 2microl after thermal denaturation and of these for 5 minutes at 95 degrees C moreover. It applies to gel and migrates. The presentation of loading liquid is the formamide solution containing Blue Dextran 0.05% which carried out deionization processing. The migration buffer solution used 1xTME (6.2). In the analysis of measurement data, it is the software Fragment Manager V1.2 (Amersham Pharmacia Biotech K.K.) for analysis. It uses.
[0037] (5) as the SSCP electrophoresis condition electrophoresis temperature of 20 degrees C, electrical-potential-difference 1500V, 100mA of currents, power 30W, and the migration buffer solution -- 1xTME (6.2) -- using it -- 500 a part -- between -- electrophoresis -- it carries out.
[0038] (6) Genotype judging PCR DNA obtained by carrying out magnification processing It is the software Fragment Manager V1.2 (Amersham Pharmacia Biotech K.K.) for analysis about the peak which carried out thermal denaturation of the fragment and was separated according to the difference of the mobility of single-stranded higher order structure. It uses and analyzes and genotype is judged. the judgment of genotype -- drawing 1 .A the case where a pattern is detected -- 148 in the 6th intron the base of eye watch -- the homozygote of a cytosine, and drawing 1 .B the case where a pattern is detected -- the heterozygote of a cytosine and a thymine, and drawing 1 .C a pattern -- detection **** -- a case -- a thymine -- it considers as a homozygote. The check of a base sequence is Thermo Sequenase Cy5 Dye Terminator Kit (Amersham Pharmacia Biotech K.K.). The used base sequence analysis performs. Under the present circumstances, 5'-TTCCATAGTGACAACTCGGG-3' is used for an oligonucleotide primer.
[0039] [Example 1] This example was performed according to each actuation of the above-mentioned experiment actuation. Adult 200 of the Japanese who chose at random About a name, it is the above-mentioned experiment actuation (1). - (6) It follows and is genomic DNA. Variation by preparation, magnification processing, removal of a superfluous primer, and SSCP was detected, and genotype was judged. The frequency of occurrence of each genotype is shown in Table 1. In addition, the 148th of the 6th intron of MASP-1 gene Allele wrote [the genotype of the homozygote whose watch base is a cytosine] TT [the genotype of TC and the homozygote which are both thymines] for the genotype of CC and the heterozygote which are a cytosine and a thymine, respectively.
[0040] [Table 1] 200 MASP-1 genotype frequency-of-occurrence analyses in healthy people CC TC TT number (frequency) 116 (58.0%) 71 (35.5%) 13 (6.5%) [0041] 58.0%, the genotype TT of the homozygote which is a thymine 35.5% is 6.5 %, therefore both the genotypes TC of the heterozygote which are a cytosine and a thymine, respectively are [the genotype CC of the homozygote whose allele is a cytosine for a result] the 148th of the 6th intron of MASP-1 gene. The variation from the cytosine in a watch base to a thymine is judged to be polymorphism.

[Translation done.]